CORRESPONDENCE

The Assessment of Serum Hepatitis C Virus RNA 12 Weeks After the End of Treatment Using TaqMan Polymerase Chain Reaction Is Less Relevant Than After 24 Weeks for Predicting Sustained Virological Response

To the Editor:

We read with great interest the article by Martinot-Peignoux et al.[1] In this report from France, undetectable serum hepatitis C virus (HCV) RNA at 12 weeks (W+12) (409 patients) post-treatment follow-up was as relevant as undetectable serum HCV RNA at 24 weeks (W+24) (sustained virological response [SVR]; 408 patients) after the end of treatment.

Current standard therapy is based on a combination of pegylated interferon (PEG-IFN) and ribavirin, but it leads to only ~50% SVR in patients with HCV genotype 1 and high viral loads.[2] IFN reduced the risk for HCC, especially among patients with SVR.[3,4] Then, we need to accurately judge whether the patient is SVR or non-SVR, applying the present standard for the judgment of SVR with the undetectability of serum HCV RNA at post-treatment W+24.

We investigated 102 patients with chronic hepatitis C genotype 1 treated with PEG-IFN-alfa 2a plus ribavirin for 48 weeks. Some of these patients had already been included in previous reports.[5,6] Serum HCV RNA was measured using the COBAS TaqMan HCV test with a detection limit of 1.2 logIU/mL. At the W+24 post-treatment follow-up, 40 (39.2%) patients had SVR, and 31 (48.4%) and 9 (23.6%) were treatment naïve and previously treated patients, respectively. At W+12, serum HCV RNA was undetectable in 42 patients, and 40 patients were SVR (PPV, 95.2%). We found two relapsers at W+24 (undetectable at W+12).

In the case of using direct-acting antivirals, earlier knowledge of treatment outcome would be useful for retreatment for the same patient. Taken together, our findings show that W+12 undetectable serum HCV RNA is not suitable for predicting persistent virological response. Further understanding of the mechanism of relapse could be useful in reducing the post-treatment follow-up period from the current standard of 24 weeks.

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Reply:

We read, with interest, the letter by Kanda et al. regarding our article. As you know, the primary end-point of antiviral therapy for chronic hepatitis C virus (HCV) is achieving sustained virological response (SVR), defined as undetectable HCV-RNA in serum 24 weeks after stopping antiviral therapy. SVR is equivalent to viral eradication and is associated with a reduction in the risk of cirrhosis and hepatocellular carcinoma.[1] Recently, it has been proposed that a 12-week post-treatment follow-up might be as relevant as 24 weeks to determine the SVR in patients with HCV receiving pegylated-interferon (PEG-IFN) and ribavirin.[2] In our study, 573 patients who received combination PEG-IFN and ribavirin and had a virological response at the end of treatment were evaluated. Serum HCV-RNA was measured, using a new assay based on transcription-mediated amplification (TMA), with a lowest detection limit of 5-10 IU/mL, at week (W)+12 and W+24 after the end of treatment. At the W+24 post-treatment follow-up, 408 (71%) patients had an SVR, 181 (71.2%) were treated with PEG-IFN alpha-2a and ribavirin, and 227 (71.1%) were treated with PEG-IFN alpha-2b and ribavirin. At W+12, serum HCV-RNA was undetectable in 409 patients, and 408 patients were SVR (positive predictive value [PPV]: 99.7%; 95% confidence interval: 99.1-100). These results show that the assessment of serum HCV-RNA 12 weeks after the end of treatment, using the highly sensitive TMA assay (PPV: 99.7%), is as relevant as after 24 weeks to predict SVR, suggesting a new definition for SVR.[2] Kandas et al. discussed these results, reporting on 2 relapsers at W+24 who were undetectable at W+12.[1] However, they used, for HCV-RNA measure, the Cobas TaqMan HCV test assay with a limit of detection of 15 UI/mL (1.2 log). In our study, we used the TMA assay, the most sensitive assay with a limit of detection lower than 5 UI/mL (0.7 log).[3-5] Our results were confirmed by an independent group.[6] Furthermore, this independent group also observed, during week 12 of follow-up, that HCV-RNA testing, using assays less sensitive, provided reliable estimates of SVR to PEG-IFN and ribavirin therapy in naïve patients.

In the future, a better understanding of mechanisms of response to PEG-IFN and ribavirin will be mandatory.[7] Also, using direct-acting antivirals, earlier knowledge of treatment outcome would be useful.[8] Our proposal for a new definition of SVR (i.e., 12-week post-treatment follow-up), studied with PEG-IFN and ribavirin treatment, will have to be assessed with a future treatment regimen.

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The Common I148 M Variant of PNPLA3 Does Not Predict Fibrosis Progression After Liver Transplantation for Hepatitis C

To the Editor:

Liver fibrosis is a complex genetic trait that is affected by multiple exogenous (i.e., environmental) and endogenous (i.e., genetic) factors. Two recent reports in Hepatology have associated a single-nucleotide polymorphism (rs738409, I148M) in the gene encoding adiponutrin/patatin-like phospholipase domain-containing 3 (*PNPLA3*) with the development of liver fibrosis and cirrhosis in hepatitis C virus (HCV)-infected patients.[1,2] In the study by Trepo et al., carriers of the *PNPLA3* rs738409 GG genotype also showed a higher rate of fibrosis progression, compared to subjects carrying wild-type alleles.[2] Liver transplantation (LT) for HCV liver disease is a special clinical setting in which fibrosis progression strongly determines the patient's prognosis. Reinfection of the graft with HCV is a universal event and leads to the accelerated development of severe (i.e., ≥F3) fibrosis in 40%-50% of patients with 5-10 years.[3]

Based on this background, we assessed whether the common *PNPLA3* I148M polymorphism would be associated with early recurrence of severe fibrosis or other clinical parameters after LT for end-stage HCV infection. In total, 176 subjects (112 male; mean age at LT, 54.4 ± 8.1 years) were included into the study who underwent protocol biopsies for the evaluation of fibrosis progression for at least 5 years during follow-up. When applying severe fibrosis recurrence in the graft as the main outcome parameter, the rs738409 genotype was not associated with the development of F3 fibrosis in the protocol biopsies at years 1, 3, and 5 after LT (Table 1). We also assessed whether the *PNPLA3* variant would be associated with hepatocellular carcinoma (HCC), acute rejections, or diverse laboratory parameters (including alanine aminotransferase, gamma-glutamyl transferase, alkaline phosphatase, bilirubin, glucose, creatinine, and International Normalization Ratio) within the first 5 years after LT. However, we could not detect any significant associations of all of these parameters with the rs738409 genotypes or alleles. Limitations of our study were that we only genotyped the recipient, but not the donor, and that we did not directly assess histological steatosis grade in the biopsies. Although we cannot exclude a contribution of the donor rs738409 genotype to the outcome after LT, the steatosis grade in the graft was dependent on various parameters (including the type of immunosuppression[4]) and might, therefore, be difficult to genetically ascertain in the post-LT setting. In summary, we conclude that, contrary to nonimmunosuppressed HCV-infected individuals, the recipient's *PNPLA3* genotype is not a strong risk factor for the outcome after LT.

Table 1. Association of rs738409 With Progression to F3 Fibrosis in Protocol Biopsies at Years 1, 3, and 5

	rs738409 CC	*rs738409 CG*	*rs738409 GG*	*P* Value
F3 at year 1	11 (6.3)	11 (6.3)	0 (0)	0.46
No F3 at year 1	77 (43.8)	60 (34.1)	17 (9.7)	
F3 at year 3	15 (8.5)	18 (10.2)	5 (2.8)	0.13
No F3 at year 3	73 (41.5)	53 (30.1)	12 (6.8)	
F3 at year 5	24 (13.6)	27 (15.3)	7 (3.9)	0.11
No F3 at year 5	64 (35.9)	44 (25.0)	10 (5.6)	

Data are given as n (%) of patients with available biopsy at indicated time. *P* value was calculated by Armitage's trend test.

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Patatin-Like Phospholipase Domain Containing-3 Ile148Met and Fibrosis Progression After Liver Transplantation

To the Editor:

Recent reports demonstrated that the *PNPLA3* (patatin-like phospholipase domain containing-3) isoleucine-to-methionine variant at residue 148 (I148M) influences steatosis and liver damage progression in chronic hepatitis C (CHC).[1-4]

The article by do O and coworkers now report that in 176 German patients with CHC who underwent liver transplantation, there was no significant effect of *PNPLA3* genotype on fibrosis after 5 years of follow-up.[5] Unfortunately, steatosis assessment was not available. Other major drawbacks limit the validity of these findings. First, previous studies have shown that the effect of *PNPLA3* on fibrosis in CHC follow a recessive model,[1-4] so that this study[5] had only 30% power to detect a two-fold increased risk. Furthermore, the effect might be less relevant in patients carrying genotype-3 hepatitis C virus, but viral features were not reported. Most importantly, the authors could only evaluate recipient genotype, so that they had no information on *PNPLA3* status in the transplanted liver. However, it is most commonly held that the 148M *PNPLA3* variant predisposes to liver damage by acting directly at the level of hepatocytes.[6] Therefore, the purported evidence does not exclude a clinically relevant role of *PNPLA3* genotype in determining the outcome of orthotopic liver transplantation for CHC, opposite to what has been suggested. Additional, adequately powered studies with systematic evaluation of steatosis, viral features, and both donor and recipient *PNPLA3* genotype are required to clarify this issue. Indeed, such a study would be of utmost importance for the following reasons: (1) it would clarify the cell type (hepatocytes versus adipocytes, or both) whose metabolic function is deranged due to *PNPLA3* variants, which has not been possible in mouse studies due to different expression pattern and mechanism of regulation of this gene, with implications for the design of new therapies, and (2) it would possibly provide useful information for organ allocation. A cooperative effort is warranted to achieve these goals.

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Potential conflict of interest: Nothing to report.

Hepatic Stellate Cell Proliferation: A Potential Role of Protein Kinase R

To the Editor:

We read with great interest the article recently published in this journal by Tarrats et al.[1] In that study the authors investigated the role of tumor necrosis factor receptors (TNFRs) 1 and 2 in hepatic stellate cell (HSC) proliferation and activation. In particular, they demonstrated that TNF-α and its receptor 1 are main players in HSC proliferation and matrix metalloproteinases-9 expression,

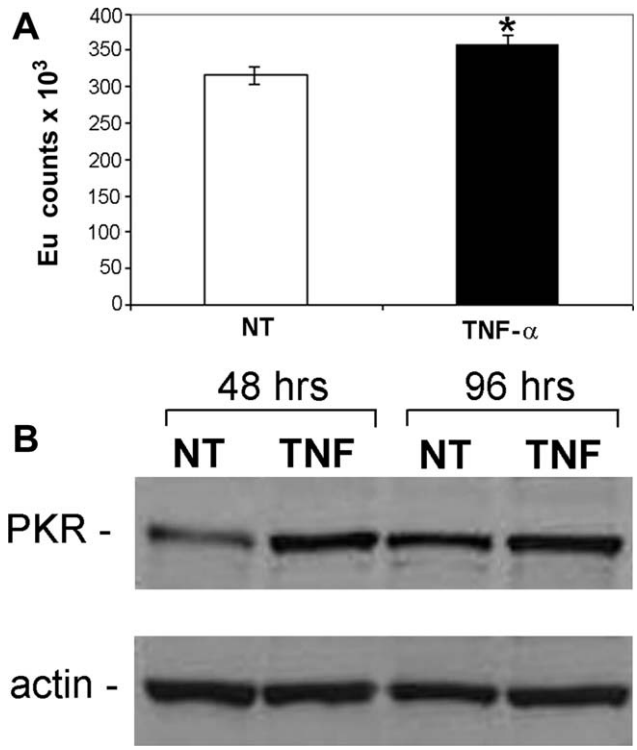

Fig. 1. TNF-α effects on proliferation and PKR protein expression in LX-2 cells. (A) Proliferation rate at 24 hours was evaluated in nontreated (NT) and treated (TNF) LX-2 as bromodeoxyuridine (BrdU) incorporation performed with Delphia kit based on Europium (Eu)-labeled antibody and Envision plate reader (Perkin Elmer, Italy; generously donated by Nicolò Valenti Onlus, Italy). Results are reported as Eu counts. $*P < 0.05$. (B) PKR expression levels were observed in total lysates (upper panel). Actin was used as loading control (lower panel).

even though both TNF-α and TNFR1 were not directly involved in HSC transdifferentiation steps generating the myofibroblast phenotype. These results were confirmed both in primary mouse HSCs and in a human HSC cell line (LX-2). Noteworthy, TNF-α appeared to be involved in the induction of the tissue inhibitor of metalloproteinase-1 only in a murine model. The authors attempted to explain this discrepancy, forgetting to highlight the fact that mouse HSC are primary, whereas LX-2 are immortalized, cells. Moreover, the lack of TNFR1 inhibited HSC proliferation only upon platelet-derived growth factor (PDGF) stimulation. The authors suggested that this effect might be mediated by PI3K/AKT signaling impairment, as well as by a direct/indirect crosstalk between TNF and PDGF receptors. It is reasonable that nuclear factor kappaB (NF-κB) upstream and downstream molecules are potential mediators of suppressed PDGF-dependent proliferation, due to the absence of functioning of TNFR1. Although these NF-κB-associated mediators still remain obscure, we believe that protein kinase R (PKR) could be a potential candidate.

It is well known that PKR is critical to cell proliferation. Specifically, it has been demonstrated that TNF-induced cell proliferation is suppressed in PKR-deficient cells.[2] In addition, PKR has been described as being involved in PDGF signaling, although its specific role has still not been elucidated.[3] Taken together, these data suggest that PKR is a possible mediator at the interface in the suggested crosstalk between PDGF and TNF receptor signaling.

We analyzed the expression and/or activation of PKR in LX-2 cells treated with TNF-α (10 ng/mL) at different timepoints. As shown in Fig. 1A, TNF-α stimulation resulted in a significant increase of HSC proliferation at 24 hours. Moreover, western blot analysis showed an up-regulation of PKR protein expression in TNF-α-treated cells at 48 hours and 96 hours (Fig. 1B).

Altogether, these results support our hypothesis that PKR might be the critical molecular link between PDGF and TNFR1 signaling pathways. The role of PKR in regulating PDGF-mediated HSC proliferation and activation, and its correlation with TNFR1, require further studies. However, the findings from the study by Tarrats et al., together with our results, add novel interesting perspectives for designing targeted molecular approaches against liver fibrogenesis.

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Potential conflict of interest: Nothing to report.

Reply:

We appreciate the comments of Dr. Ceccarelli and colleagues[1] regarding our article "Critical role of tumor necrosis factor receptor 1, but not 2, in hepatic stellate cell proliferation, extracellular matrix remodeling, and liver fibrogenesis,"[2] and its suggestion of the potential participation of protein kinase R (PKR) at the crosstalk between tumor necrosis factor receptor 1 (TNFR1) and platelet-derived growth factor-β (PDGF-β) receptor in hepatic stellate cells (HSCs). In fact, in light of the background information provided and their observation that PKR expression is boosted by TNF in LX2 cells, we have decided to pursue this line of investigation by analyzing the basal protein expression of PKR in our TNFR1 knockout (TNFR1-KO) HSCs, TNFR1/TNFR2 double-knockout (TNFR-DKO) HSCs, and wild-type HSCs to validate if differences in PKR expression could account for the lack of AKT activation and proliferation observed after PDGF challenge in TNFR1-KO and TNFR-DKO HSCs.[2] We did not observe differential expression in PKR protein expression between wild-type, TNFR1-KO, and TNFR-DKO HSCs (Fig. 1). Although these initial observations do not support a critical role for PKR in the proliferative effects of TNF in murine HSCs, recent observations indicate that PKR undergoes rapid phosphorylation following the engagement of TNF receptors by TNF.[3] Therefore, future analyses of PKR phosphorylation, rather than its total expression, is warranted to critically examine whether PKR participates in the

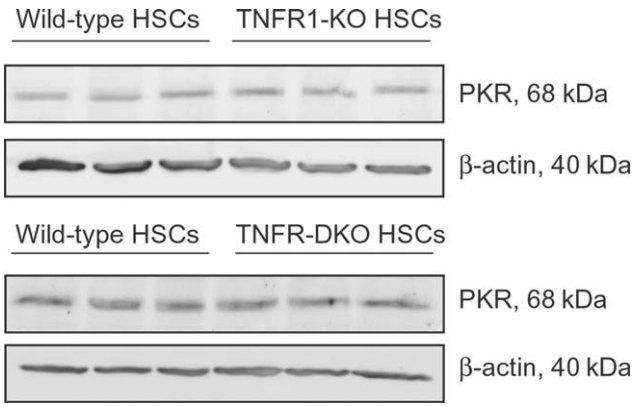

Fig. 1. Protein kinase R (PKR) expression in 7-day-old wild-type, TNFR1 knockout (TNFR1-KO) and TNFR1/TNFR2 double-knockout (TNFR-DKO) HSCs. Samples were run in triplicate. Antibodies used were monoclonal anti-PKR (clone B-10), anti–mouse–horseradish peroxidase (HRP) (Santa Cruz Biotechnology), anti–β-actin–HRP (Sigma-Aldrich).

proliferation of HSCs by TNF. In addition, whether PKR activation is differentially involved in human LX2 cells, as described by Ceccarelli et al.,[1] versus murine HSCs (our present observations) deserves further investigation. Alternatively, besides PKR, other unknown intermediates involved in nuclear factor-κB activation may also participate in the cross-talk between TNF and PDGF signaling. Although described in a different context, recent observations have uncovered a new role for Sam68, a member of the signal transducers and activators of RNA (STAR) family of proteins that regulate RNA processing, in the TNFR signaling complexes.[4] Nevertheless, we fully agree with Ceccarelli et al. that further investigations to identify intermediates controlling HSC proliferation and/or activation in response to TNF and PDGF may be of significant value in the treatment of liver fibrosis.

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Raised Sympathetic Activity and Blood Ammonia Could Increase Sensitivity to Infections in Patients with Cirrhosis and Refractory Ascites Who Are Using Beta-Blockers

To the Editor:

We read with interest the study by Sersté et al. who found a negative impact of beta-blockers on survival in patients with cirrhosis and refractory ascites.[1] Interestingly, 79.5% of patients with a specified cause of death died of sepsis, suggesting an association between chronic use of beta-blockers and development of bacterial infections, yet the authors did not provide an explanation. We suggest that raised blood norepinephrine (NE) and ammonia levels could contribute to increased vulnerability to bacterial infections in patients with refractory ascites who are taking beta-blockers.

Circulating NE levels can be increased in cirrhosis due to reduced effective blood volume, impaired liver metabolism, portosystemic shunts, and propranolol treatment.[2,3] Patients with refractory ascites are commonly characterized by markedly increased plasma NE levels.[2] High NE levels have been shown to suppress neutrophil chemotaxis[4] and phagocytosis[5] as well as to inhibit macrophage proliferation and promote their apoptosis.[6] In the study of Sersté et al., beta-blocker–treated patients had worse liver function and systemic hemodynamics, which together with the presence of collaterals and propranolol treatment could result in higher NE concentration compared to the nontreatment group. Blood ammonia can also impair neutrophil phagocytosing function in cirrhosis[7] and increases significantly with the grade of liver disease severity and esophageal varices,[8] and after propranolol administration.[9] Consequently, blood ammonia levels could be higher in the group treated with beta-blockers, which matches with the higher incidence of encephalopathy in these patients. We conclude that data regarding blood NE and ammonia concentration would be useful for the interpretation of the observations of Sersté et al.

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Metabolic Syndrome and Liver Cancer: is Excess Iron the Link?

To the Editor:

Welzel et al.[1] found that preexisting metabolic syndrome conferred a statistically significant increase of primary liver cancers that was independent of other risk factors. We suggest that this pathological association may partially be related to the higher body iron stores often found in such patients.

A wealth of evidence has established a link between serum ferritin, insulin resistance, and nonalcoholic fatty liver disease (NAFLD). Body iron excess has frequently been found in patients with metabolic syndrome.[2] Furthermore, it has been suggested that the relation between serum ferritin and most of metabolic syndrome features might be mediated by the presence of NAFLD at the population-based level.[3] Excessive hepatic iron accumulation in NAFLD can be one of the potential cofactors involved in enhanced oxidative stress, which triggers liver cell necrosis and activation of hepatic stellate cells, both of which lead to fibrosis.[4] Indeed, iron depletion by phlebotomy was found to be beneficial in improving insulin resistance in patients with NAFLD and hyperferritinemia.[5]

On the other hand, it has been shown that individuals with excess total body iron have a higher risk of liver cancer even in the absence of genetic hemochromatosis.[6] Interestingly, iron depletion therapy with both phlebotomies and a low-iron diet was shown to significantly lower the risk of hepatocellular carcinoma in patients with chronic hepatitis C.[7]

Therefore, we hypothesize that iron, metabolic syndrome, NAFLD, and liver cancer may be linked together, and their risk might be modified in parallel by maneuvers that affect either feature.

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Absence of Occult Hepatitis C Virus Infection in Patients Under Immunosupressive Therapy for Oncohematological Diseases

To the Editor:

We read with interest the review by Welker and Zeuzem[1] on occult hepatitis C virus (HCV) infection and replies by Carreño et al.[2] and Halfon et al.[3] and would like to make our contribution to this topic regarding precisely the role of occult HCV infection in immune-compromised patients. Recently, Barrill et al. found that 45% of 109 anti-HCV–negative hemodialysis patients with abnormal serum aminotranferases had HCV RNA in peripheral blood mononuclear cells (PBMCs),[4] but no data are so far available on occult HCV infection in oncohematological patients.

We prospectively enrolled 28 consecutive anti-HCV–negative patients with an oncohematological disease who first underwent chemotherapy from April 2006 to November 2007. All patients were screened for hepatitis B surface antigen (HBsAg), anti-HBs (antibody to hepatitis B surface antigen), anti-HBc (antibody to hepatitis B core antigen), and anti-HCV. The diagnosis and

treatment of the oncohematological diseases were based on commonly accepted criteria.

For each patient, samples of plasma and PBMCs were obtained at enrollment, at months 1 and 3 during chemotherapy, and then every 3 months after treatment discontinuation. The 28 patients were treated with chemotherapy for 4-12 months and observed after its discontinuation for 6-24 months. PBMCs were isolated from 5 mL whole blood by means of Histopaque (Sigma-Aldrich, St. Louis, MO) according to a standard technique and collected in aliquots of 2×10^6 cells. The presence of HCV RNA in plasma and PBMCs of all samples collected during the study was determined as previously reported.[5] The detection limit in the plasma samples was around 40 IU/mL. The sensitivity of our method to detect HCV RNA in PBMC samples was assessed using HCV-positive PBMCs diluted in PBMCs obtained from an HCV RNA–negative patient, as described by Halfon et al.[6] Briefly, 2×10^6 PBMCs from an HCV RNA–positive patient quantified at 1.8×10^4 IU/2×10^6 PBMCs was sequentially diluted (1:10) in 2×10^6 HCV RNA–negative PBMCs; in these PBMC mixtures, HCV RNA was then quantified by real-time polymerase chain reaction. The lowest detection limit by this method was 18 IU/2×10^6 cells. As a positive control for extraction of RNA from PBMCs, glucose-6-phosphate dehydrogenase (G6PDH) messenger RNA was sought in all PBMC samples collected (LightCycler h-G6PDH Housekeeping Gene Set; Roche Diagnostics, Branchburg, NJ).

Table 1. Demographic, Clinical, and Serological Characteristics of the 28 Anti-HCV-Negative Patients

Characteristic	Anti-HCV–Negative Patients
Number of patients	28
Age, years (mean ± SD)	64.1 ± 8.52
Males, n (%)	16 (57.1)
Hematological diseases:	
B-NHL, n (%)	16 (57.1)
HL, n (%)	1 (3.6)
CLL, n (%)	6 (21.4)
Multiple myeloma, n (%)	5 (17.9)
Type of immunosuppressive therapy	
Rituximab, n (%)	2 (7.15)
R-CHOP, n (%)	14 (50)
Chlorambucil + methylprednisolone, n (%)	4 (14.3)
Fludarabine + prednisone, n (%)	1 (3.6)
Fludarabine + cyclophosphamide, n (%)	0
R-FC, n (%)	1 (3.6)
Bortexomib + dexamethasone, n (%)	2 (7.2)
ABVD, n (%)	1 (3.6)
DVD, n (%)	2 (7.2)
	1 (3.6)
Melphalan + prednisone, n (%)	
Aspartate aminotransferase, n.v.x (mean ± SD)	0.66 ± 0.25
Alanine aminotransferase, n.v.x (mean ± SD)	0.8 ± 0.33
Total bilirubin, mg/dL (mean ± SD)	0.68 ± 0.27
% Prothrombin activity, median (range)	98 (70-118)
Serological status for HBV, n (%)	
HBsAg+	3 (10.7%)
HBsAg−/anti-HBs+/anti-HBc+	11 (39.3%)
HBsAg−/anti-HBs−/anti-HBc+	5 (17.9%)
HBsAg−/anti-HBc−	9 (32.1%)
Anti-HBV prophylaxis/therapy, n (%)	
Lamivudine	16 (57.1%)
Telbivudine	2 (7.1%)
Entecavir	1 (3.6%)
None	9 (32.1%)

ABVD, doxorubicin, bleomycin, vinblastine, dacarbazine; CHOP, rituximab, cyclophosphamide, doxorubicin, vincristine, prednisone; CLL, chronic lymphocyte leukemia; DVD, pegylated liposomal doxorubicin, vincristine, dexamethasone; HL, Hodgkin lymphoma; NHL, non-Hodgkin lymphoma; n.v. x, normal value x; R-FC, rituximab, fludarabine, cyclophosphamide; SD, standard deviation.

Table 1 shows the demographic, clinical, biochemical, and serological characteristics observed at the baseline in the 28 patients enrolled (Table 1). The three HBsAg-/HBV DNA–positive patients at the baseline were treated with telbivudine or entecavir. They became HBV DNA–negative within 6 months while still under treatment and remained so throughout the observation; the 16 HBsAg-negative/anti-HBc–positive patients received lamivudine prophylaxis and never showed circulating HBsAg or HBV DNA. No plasma or PBMC sample collected during the study was HCV RNA–positive. All PBMC samples collected were positive for G6PDH messenger RNA.

No patient in the present study became positive for HCV RNA in plasma or PBMCs while under chemotherapy for an oncohematological disease. The data from this longitudinal study run counter to the existence of occult HCV infection in patients under strong immunosuppression, who constitute a suitable model of investigation to explore occult HCV infection. In addition, 60.7% of the patients enrolled received rituximab-based chemotherapy, which has been demonstrated as able to increase the HCV replication in anti-HCV–positive patients.[7]

In conclusion, neither occult HCV infection nor its reactivation under strong immunosuppressive chemotherapy were found in the present study in oncohematological patients who were anti-HCV- and HCV RNA–negative. Our data and those of others[6,8] suggest the nonexistence of occult HCV infection.

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Potential conflict of interest: Nothing to report.

Vitamin D Levels May Explain the Racial Differences in Response Rates to Antiviral Therapy for Chronic Hepatitis C

To the Editor:

Despite many studies that report black patients infected with chronic hepatitis C virus genotype 1 show lower rates of sustained virologic response than nonblacks to treatment with peginterferon plus ribavirin,[1,2] the underlying reasons for the racial differences in response rates to antiviral therapy remain obscure. I read with great interest the article by Petta et al.,[3] in which the authors reported that low vitamin D serum level is related to low responsiveness to antiviral therapy in individuals chronically infected with hepatitis C genotype 1, and lower 25-hydroxy vitamin D (25(OH)D) serum level is an independent negative risk factor for sustained virologic response. I think this finding has important implications for understanding the racial differences in response rates to antiviral therapy of chronic hepatitis C.

Vitamin D levels vary in individuals of different ethnicity. Because the higher amount of pigmentation in their skin reduces vitamin D production by sunlight, blacks have been well documented to have lower vitamin D levels than that of nonblacks, and vitamin D insufficiency is more prevalent among black Americans than nonblack Americans. A cross-sectional analysis of serum 25(OH)D levels in black and white subjects enrolled in the Southern Community Cohort Study indicated that hypovitaminosis D prevalence was 45% among blacks and only 11% among whites.[4] According to the finding of Petta et al. that lower 25(OH)D serum level is an independent negative risk factor for sustained virologic response for chronic hepatitis C genotype 1,[3] it is reasonable to infer that the lower vitamin D levels in blacks may make them respond less well to antiviral therapy with peginterferon and ribavirin than do nonblacks.

Thus, besides the decreased prevalence among blacks of an interleukin-28B gene polymorphism associated with interferon responsiveness,[5] the differences in vitamin D status among blacks and nonblacks may also contribute to the lower response rate in blacks to the antiviral treatment with peginterferon and ribavirin. Moreover, examination whether vitamin D supplementation can increase the rates of antiviral therapy response for patients, especially for blacks, infected with chronic hepatitis C virus deserves further investigation.

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Postoperative Transcatheter Arterial Chemoembolization Should Be Recommended in the Hepatocellular Carcinoma Treatment Guidelines of the American Association for the Study of Liver Diseases

To the Editor:

We read with great interest the updated guidelines on hepatocellular carcinoma (HCC) by the American Association for the Study of Liver Diseases.[1] The guidelines suggest that pre- or postresection adjuvant therapy is not recommended.[1] As the guidelines recommend, it is suggested that preoperative transcatheter arterial chemoembolization (TACE), worsens overall survival (OS) rate and increases the risk of HCC recurrence for resectable HCC.[2] As a result, they propose that preoperative TACE cannot be recommended as a routine procedure before hepatectomy for a resectable HCC, in accordance with our opinion.

However, for the postoperative period, guidelines ignore a significant amount of data about the use of TACE. Recently, accumulating evidence has demonstrated that patients with HCC can benefit from postoperative TACE. A prospective randomized trial in patients with stage IIIA HCC (clinical trial NCT00652587), recruited 115 patients with stage IIIA HCC to undergo hepatectomy with adjuvant TACE or to undergo hepatectomy alone.[3] The 1-, 3-, and 5-year OS rates and median OS for hepatectomy with

adjuvant TACE were 80.7%, 33.3%, 22.8%, and 23.0 months, respectively, and for hepatectomy alone were 56.5%, 19.4%, 17.5%, and 14.0 months, respectively ($P = 0.048$). The 1-, 3-, and 5-year disease-free survival (DFS) rates and median DFS for hepatectomy with adjuvant TACE were 29.7%, 9.3%, 9.3%, and 6.0 months, respectively, and for hepatectomy alone were 14.0%, 3.5%, 1.7%, and 4.0 months, respectively ($P = 0.004$). Thus, for patients with stage IIIA HCC, hepatectomy with adjuvant TACE efficaciously and safely improved survival outcomes when compared with hepatectomy alone. More importantly, a meta-analysis including six randomized controlled trials totaling 659 participants were included.[4] For the 1-year tumor recurrence rate, hepatectomy plus TACE showed statistically significant less incidence of recurrence, with a pooled relative risk (RR) of 0.68 (95% confidence interval [CI] = 0.55-0.84, $P = 0.0003$). For 1- and 3-year mortality, the trials were favorable for TACE with a pooled RR of 0.48 (95% CI = 0.35-0.65, $P < 0.00001$) and with a pooled RR of 0.76 (95% CI = 0.64-0.90, $P = 0.002$). Therefore, when used as a postoperative treatment, TACE could decrease tumor recurrence and improve survival for the participants with HCC with risk factors.

Taken together, because TACE is the most effective adjuvant therapy for treatment of HCC, it should be complemented in the postoperative period for HCC. Thus, it seems plausible to infer that postoperative TACE should be recommended in the HCC treatment guidelines of the American Association for the Study of Liver Diseases.

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Potential conflict of interest: Nothing to report.

Genetic Variation in Interleukin-28B Locus is Associated with Spontaneous Clearance of HCV in Children with Non-1 Viral Genotype Infection

To the Editor:

In a recent article in Hepatology, Ruiz-Extremera et al.[1] reported that interleukin (IL)-28B C/C genotype in the child was associated with spontaneous clearance of hepatitis C virus (HCV) genotype 1 infection. This result is important, as, although on a limited number of patients (n = 15), the IL-28B C/C genotype is the first and only predictor of the outcome of HCV genotype 1 infection identified in children. So far, previous studies in natural history settings demonstrated only that genotype 3 was associated with a higher rate of spontaneous viral clearance in children, when compared with other genotypes.[2,3]

Our aim was, therefore, to examine the association between polymorphisms in the IL-28B gene and clearance of HCV in a cohort of children infected with an HCV non-1 viral genotype. We genotyped a total of 28 (male/female = 15/13) Caucasian children at the Pediatric and Liver Unit of the Meyer Children's University Hospital of Florence, Italy, for the single nucleotide polymorphism rs12979860 of the IL-28B gene. This cohort included 24 children with persistent infection (HCV RNA/anti-HCV positive) and 4 individuals who naturally cleared the virus (HCV RNA negative, anti-HCV positive). All the children were hepatitis B antigen and anti-human immune deficiency negative and acquired HCV infection vertically.[4] Patients with the C/C genotype were more likely to clear HCV relative to patients with the C/T and T/T genotypes combined (odds ratio = 15; 90% confidence interval = 1.2-376 ; $P = 0.04$; Table 1).

Table 1. Effect of Interleukin-28B rs12979860 Genotype on Clearance of Hepatitis C Virus in Children Infected With Non-1 Viral Genotype

Child's Interleukin-28B Genotype	Frequency of Clearance (%)	Frequency of Persistence (%)	*P* Value
C/C	3 (43)	4 (57)	0.04
C/T + T/T	1 (5)	20 (95)	

The present results strengthen the primary role for IL-28B in the resolution of HCV infection. The IL-28B C/C genotype has been associated in adults with sustained virological response to HCV drug treatment and with spontaneous clearance of HCV. The present data demonstrate that IL-28B promotes the spontaneous clearance of HCV independently of age and genotype. IL-28B polymorphisms in children treated with the recently approved combined therapy could be important in the treatment decision-making process. The independent role of IL-28B C/C genotype and viral genotype 3 in predicting spontaneous viral clearance should be evaluated in future studies enrolling a higher number of patients.

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